<Preliminary>Molecular Cloning of cDNAs of the Genes Expressed in Differentiating Xylem of Tension Wood Formation in Eucalyptus camaidulensis L.

AUTHOR(S):

TOYOTA, Jun-ichiro; BABA, Kei'ichi; HIBINO, Takashi; ITOH, Takao

CITATION:

TOYOTA, Jun-ichiro ...[et al]. <Preliminary>Molecular Cloning of cDNAs of the Genes Expressed in Differentiating Xylem of Tension Wood Formation in Eucalyptus camaidulensis L.. Wood research : bulletin of the Wood Research Institute Kyoto University 1997, 84: 31-33

ISSUE DATE:

1997-09-30

URL:

http://hdl.handle.net/2433/53191

Preliminary

Molecular Cloning of cDNAs of the Genes Expressed in Differentiating Xylem of Tension Wood Formation in *Eucalyptus camaidulensis* L.*[1]

Jun-ichiro TOYOTA*[2], Kei'ichi BABA*[2],
Takashi HIBINO*[3] and Takao ITOH*[2]

(Received May 31, 1997)

Keywords: cell wall, *Eucalyptus*, gene expression, tension wood, xylem

Introduction

Tension wood is a reaction wood formed in a leaning stem of angiosperm. Reaction wood causes to bend the stem upwards by their mechanical action. Tension wood formation is stimulated by the change of gravity direction to the stem axis[1)], however, the mechanism of formation is still unclear. To understand molecular-biological basis on tension wood formation, gene expression was monitored by differential screening, and some cDNA was cloned. Tension wood of *Eucalyptus camaldulensis* has been already characterized and it showed typical characteristics of tension wood as follows; high cellulose and low lignin contents, presence of G-fiber, vessels reduced in size and number, microfibrillar orientation of G-layer nearly parallels fiber axis[2)].

Materials and Methods

A stem of a three-years-old *Eucalyptus camaldlensis* L. was inclined and fixed for 2 weeks. After the stem was cut and bark was peeled, differentiating xylem of upper and lower side was collected, respectively, and immediately put them into liquid nitrogen. They were stored at −80°C until use. For negative control, differentiating xylem tissue of the stem growing vertically was also harvested in the same manner.

Total RNA was isolated from the stored tissue of upper side[3)], then poly(A)$^+$ RNA was purified by using oligo-dT cellulose column. Double-stranded cDNA was synthesized from

*[1] A part of this work was presented at the 46th Annual meeting of Japan Wood Research Society in Kumamoto (April, 1996) and was supported by Grant-in-Aid for Encouragement of Young Scientists (No. 07856017) from the Ministry of Education, Science and Culture of Japan.
*[2] Laboratory of Cell Structure and Function.
*[3] Forestry Research Institute, Oji Paper Co. Ltd., Kameyama, Mie.

the poly(A)$^+$ RNA, and was ligated into λgt 11 phage vector by using Timesaver^{TM} Kit (Pharmacia Biotech).

Subtracted probe, which was labeled with ^{32}P, was prepared from poly(A)$^+$ RNA of upper side and vertical stem by using Subtractor kit (Invirogen). A cDNA library of λgt 11 phage was screened with this probe.

The sizes of cDNA clones were measured by electrophoresis of their PCR products. The expression of them was analyzed by Northern blotting against RNAs obtained from upper side, lower side and negative control. Probes were prepared and the signals were detected by using Gene Images Kit (Amersham).

Results and Discussion

A cDNA library of differentiating xylem of tension wood was constructed within λgt 11 phage vector. The percentage of transformation was 99.8%, and the titer was 36,000 pfu/ml. The results of molecular cloning of cDNA was summarized in Table 1. Differential screening was performed on about 25,000 clones. Many positive clones occurred but 48 obvious clones were selected at the first screening. After second screening of this cDNA library with subtracted probe, 31 clones of cDNA were obtained. Insertion lengths of these clones were analyzed by electrophoresis of their PCR products. Single band insertion was found in 27 clones. The expression of these 27 clones were analyzed by Northern blotting. Within these clones, 15 clones were able to be detected their mRNA bands. They were able to be classified to 5 categories (Table 2). Transcription to mRNA occurred in (1) only upper side, (2) both side but the signal of upper side is stronger than that of lower side and not found in control, (3) all conditions but the signal of upper side is stronger than the others, (4) all conditions but the signal of upper side and lower side is stronger than that of control, (5) all conditions in similar strength. cDNA clones which were classified to (1), (2) and (3) would be possible to be involved in tension wood formation.

Table 1. Positive clones on each step of molecular cloning

Cloning steps	Number of positive clones
1st screening	48
2.d screening	31
PCR	27
Northern blot	15

Table 2. Expression pattern of mRNA detected by Northern blot

Category	Signal Strength			Number of cDNA clones
	C	L	U	
1	−	−	++	2
2	−	+	++	3
3	+	+	++	8
4	+	++	++	1
5	++	++	++	1

C: control, L: lower side, U: upper side.

References

1) W.A. CÔTÉ JR. and A.C. DAY: "Cellular ultrastructure of woody plants", W.A. CÔTÉ, JR. ed., Syracuse University Press, p. 391–418 (1965).
2) K. BABA, T. ONA, K. TAKABE, T. ITOH and K. ITO: *Mokuzai Gakkaishi*, **42**, 795–798 (1996).
3) K. BABA and K. Ito: *Wood Res.*, **84**, 7–11 (1997).